## This Page Is Inserted by IFW Operations and is not a part of the Official Record

## **BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

## IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents will not correct images, please do not report the images to the Image Problem Mailbox.





## WE CLAIM:

- 1. A method of inducing at least one site-directed doublestrand break in DNA of a cell, said method comprising
- (a) providing cells containing double-stranded DNA, wherein said DNA comprises at least one I-Sce I restriction site;
- (b) transfecting said cells with at least a plasmid comprising DNA encoding the I-Sce I meganuclease; and
- (c) selecting cells in which at least one double-strand break has been induced.
- 2. The method of claim 1, wherein said cell is selected from the group consisting of a mammalian cell, a yeast cell, and a plant cell.
- 3. The method of claim 2, wherein said cell is an NIH3T3 cell containing the G-MtkPL virus.
- 4. The method of claim 1, wherein said plasmid is  $pCMV(I-Sce\ I+)$ .
- 5. A method of inducing homologous recombination between chromosomal DNA of a cell and exogenous DNA added to said cell, said method comprising
- (a) providing cells containing chromosomal DNA, wherein said DNA comprises at least one I-Sce I restriction site;
- (b) transfecting said cells with a plasmid comprising exogenous DNA, and with a plasmid comprising DNA encoding the I-Sce I meganuclease; and
- (c) selecting cells in which said exogenous DNA is inserted into said chromosomal DNA.





- 6. The method of claim 5, wherein said cell is selected from the group consisting of a mammalian cell, a yeast cell, and a plant cell.
- 7. The method of claim 6, said cell is an NIH3T3 cell containing the G-MtkPL virus.
- 8. The method of claim 5, wherein said plasmid is pCMV(I-Sce I+).
- 9. A method of inducing homologous recombination between chromosomal DNA of a cell and exogenous DNA added to said cell, said method comprising
  - (a) providing cells comprising chromosomal DNA;
- (b) inserting at least one I-Sce I restriction site in said chromosomal DNA;
- (c) transfecting said cells with a first plasmid comprising exogenous DNA, and with a second plasmid comprising DNA encoding the I-Sce I meganuclease; and
- (d) selecting cells in which said exogenous DNA is inserted into said chromosomal DNA.
- 10. The method of claim 9, wherein said cell is selected from the group consisting of a mammalian cell, a yeast cell, and a plant cell.
- 11. The method of claim 9, wherein said first plasmid is pCMV(I-Sce I+).
- 12. The method of claim 9, wherein said second plasmid is pVRneo.
- 13. A method of inducing at least one site-directed break in DNA of a cell and inserting DNA encoding a polypeptide, said method comprising,

- (a) providing cells containing double-stranded DNA, wherein said cells are capable of being transformed by a DNA comprising a I-Sce I restriction site and DNA encoding said polypeptide;
- (b) adding Sce I enzyme or transforming said cell with DNA encoding Sce I\_enzyme;
- (c) transfecting said cells with said DNA encoding said polypeptide or with a vector containing said DNA; and
- (d) selecting cells transfected with said DNA or said vector, wherein said cells express said polypeptide.
- 14. A recombinant eukaryotic cell transformed by the method of any one of claims 1 and 13.
- 15. A transgenic animal comprising a cell transformed by the method of any one of claims 1 and 13.
- 16. A method of expressing a polypeptide in a transgenic animal, said method comprising transforming embryonic stem cells with a DNA comprising a I-Sce I restriction site and DNA encoding said polypeptide, and detecting expression of said polypeptide in a transgenic animal resulting from said transformed embryonic stem cells.
- 17. A recombinant stem cell expressing a polypeptide, wherein said stem cell is transformed by a DNA comprising a I-Sce I restriction site and DNA encoding said polypeptide by
- (a) adding Sce I enzyme to said cell or transforming said
  cell with a vector containing the gene coding for Sce I enzyme;
- (b) transfecting said cells with said DNA encoding said polypeptide; and
- (c) selecting cells transfected with said DNA, wherein said cells express said polypeptide.



- 18. A recombinant eukaryotic cell as claimed in any one of claims 4 and 7 wherein said polypeptide is a foreign antigen to the cell.
- 19. The recombinant eukaryotic cell as claimed in claim 14 wherein cell is a mammalian cell line.
- 20. The recombinant eukaryotic cell as claimed in claim 14 wherein cell is a yeast.
- 21. A method of inducing at least one site-directed break in DNA of cells and inserting DNA encoding a polypeptide, wherein said cells express at least one protein product, said method comprising,
- (a) providing cells containing double-stranded DNA, wherein said cells are capable of being transformed by a DNA comprising a I-Sce I restriction site and DNA encoding said polypeptide;
- (b) adding Sce I enzyme to said cells or transforming said cells with DNA encoding Sce I enzyme;
- (c) transfecting said cells with said DNA encoding said polypeptide or with a vector containing said DNA; and
- (d) selecting cells transfected with said DNA or said vector, wherein said cells express said polypeptide and do not express said protein product.
- 22. A recombinant cell transformed by the method of claim 21.